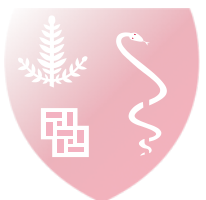


VITREOUS PROTEOMICS CORRELATES WITH GENE EXPRESSION PROFILE OF UVEAL MELANOMA

RETINA SOCIETY 2020

Prithvi Mruthyunjaya, MD, MHS, Gabriel Velez BS,
Teja Chemudupati BS, Huy V. Nguyen MD,
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*Ocular Oncology and Vitreoretinal Surgery Service
Byers Eye Institute
Stanford University*



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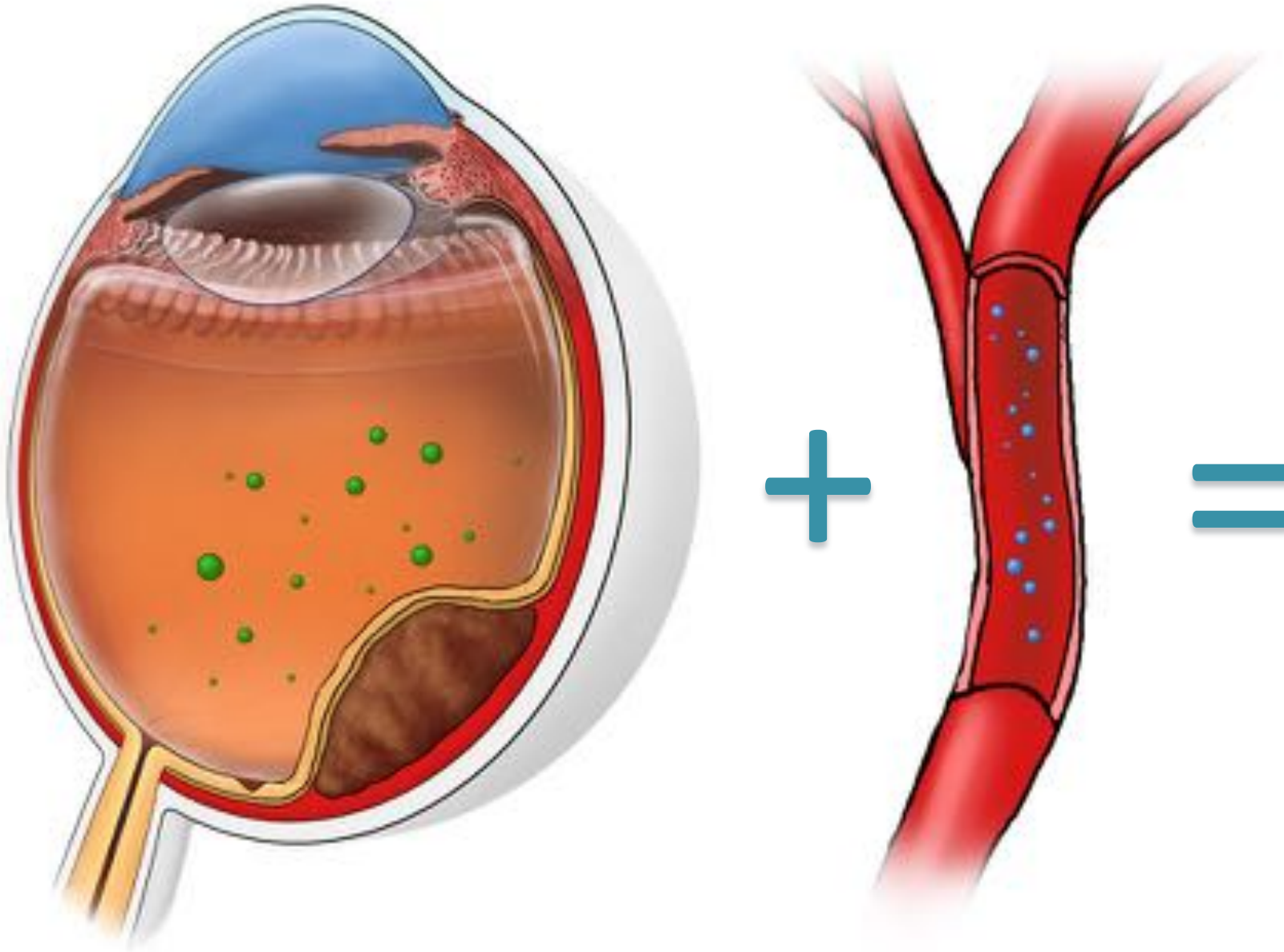
ACKNOWLEDGEMENTS AND DISCLOSURES

Disclosures

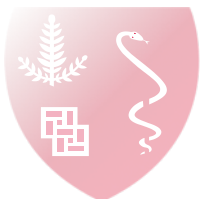
- PM: Castle Biosciences, Aura Biosciences, Arix Biosciences
- VBM: X-37, Takeda, Retinagenix, MantraBio, Ayoxxa, Protagonist, Spark Therapeutics, Guidepoint, 23&Me, Regeneron, Syncona, Vistra, Johnson & Johnson, GLG, Gyroscope, MeiraGTX



SUMMARY: PROTEOMICS APPROACH TO UM



- Detection without direct tumor biopsy
- GEP and PRAME based New targets
- Repurposed therapies



METASTATIC DISEASE IS STILL THE PROBLEM

- Up to 50% of Uveal Melanoma patients will develop metastatic disease
 - No change in survival
 - No approved or adjuvant therapy (sunitinib)



Metastatic disease from uveal melanoma:
treatment options and future prospects

Richard D Carvajal,¹ Gary K Schwartz,¹ Tongalp Tezel,² Brian Marr,³
Jasmine H Francis,³ Paul D Nathan⁴

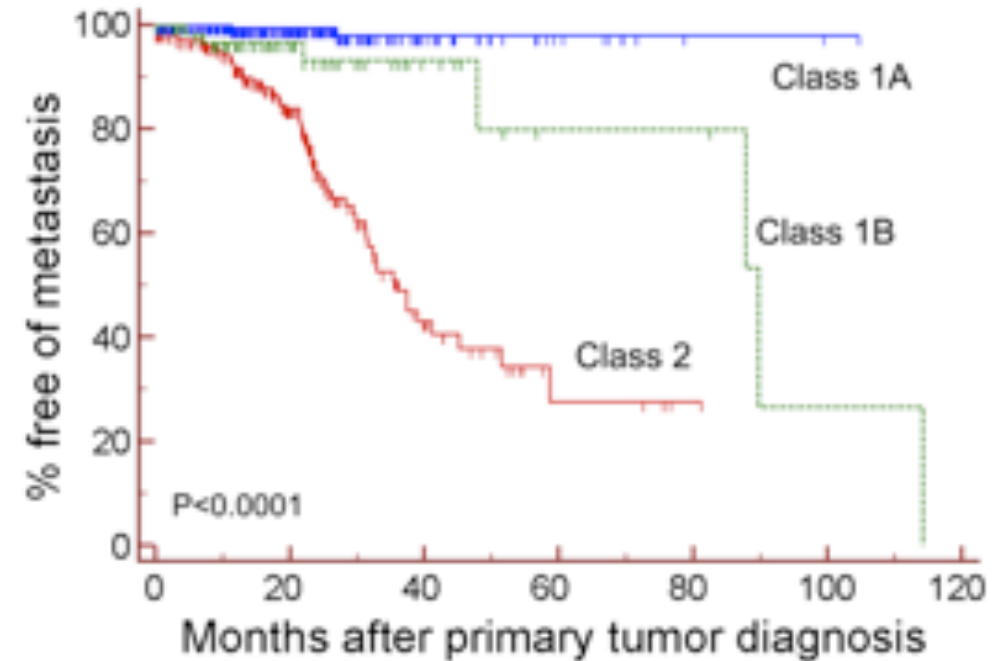
Br J Ophthalmol 2017;**101**:38–44.



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ESTIMATING METASTATIC RISK IS IMPORTANT

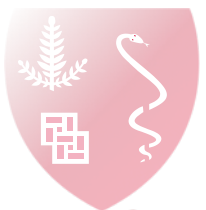
- Clinical features
- Histopathologic features
- Chromosomal alterations
 - High risk: 1p-, 6p-, +8q, -3
 - Low risk: +6p, 9p-
- *Gene expression profiling*
- *PRAME expression*



JUST BECAUSE I KNOW I HAVE A CLASS 2 MELANOMA..

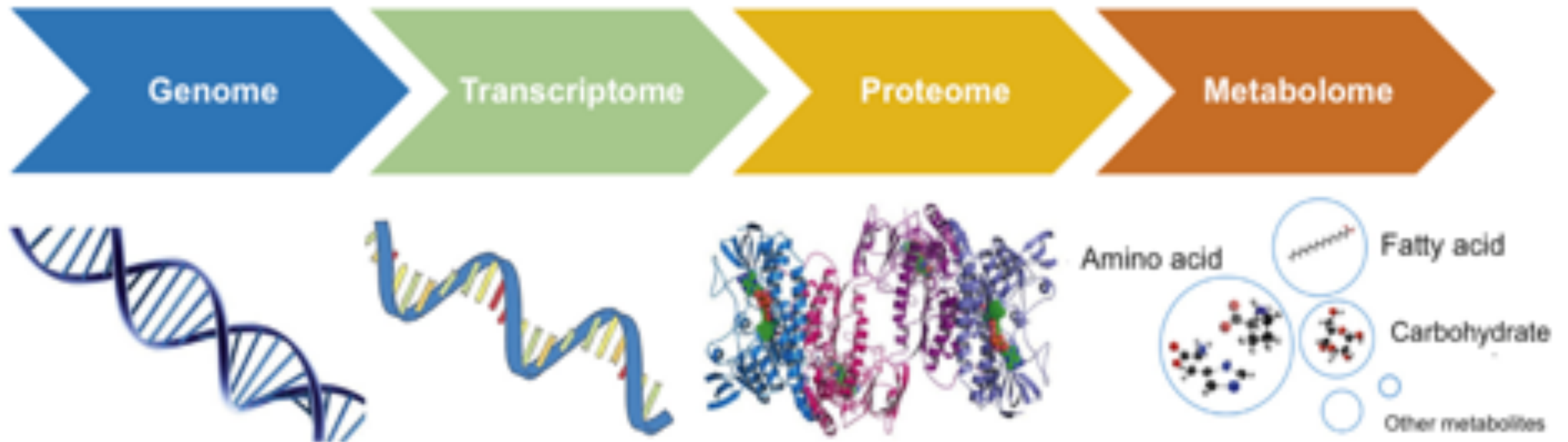
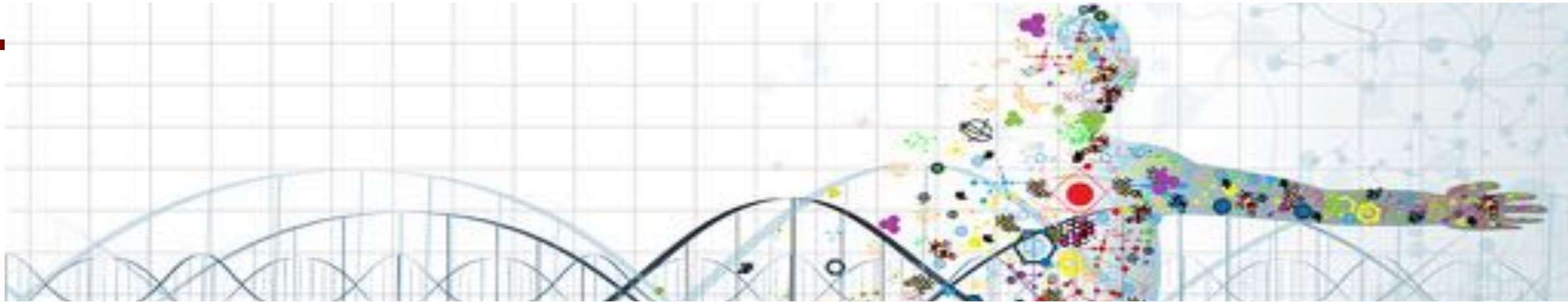
- Unable to detect micrometastatic disease in the blood
 - *Circulating tumor cells in 34-46%*
 - *CTCs in 58% and cfDNA in 26%*
- No FDA or clinically actionable agents to treat

Anand et al. Cancers 2019,
Beasley et al. JCO Precision Oncology 2018



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FROM GENOMICS TO PROTEOMICS



UVEAL MELANOMA PROTEOMICS @ STANFORD

Central hypothesis

Eye fluid and serum protein signatures can provide early targeting of metastatic risk in UM



Dr. Vinit Mahajan



PROTEOMICS IN UVEAL MELANOMA

PURPOSE:

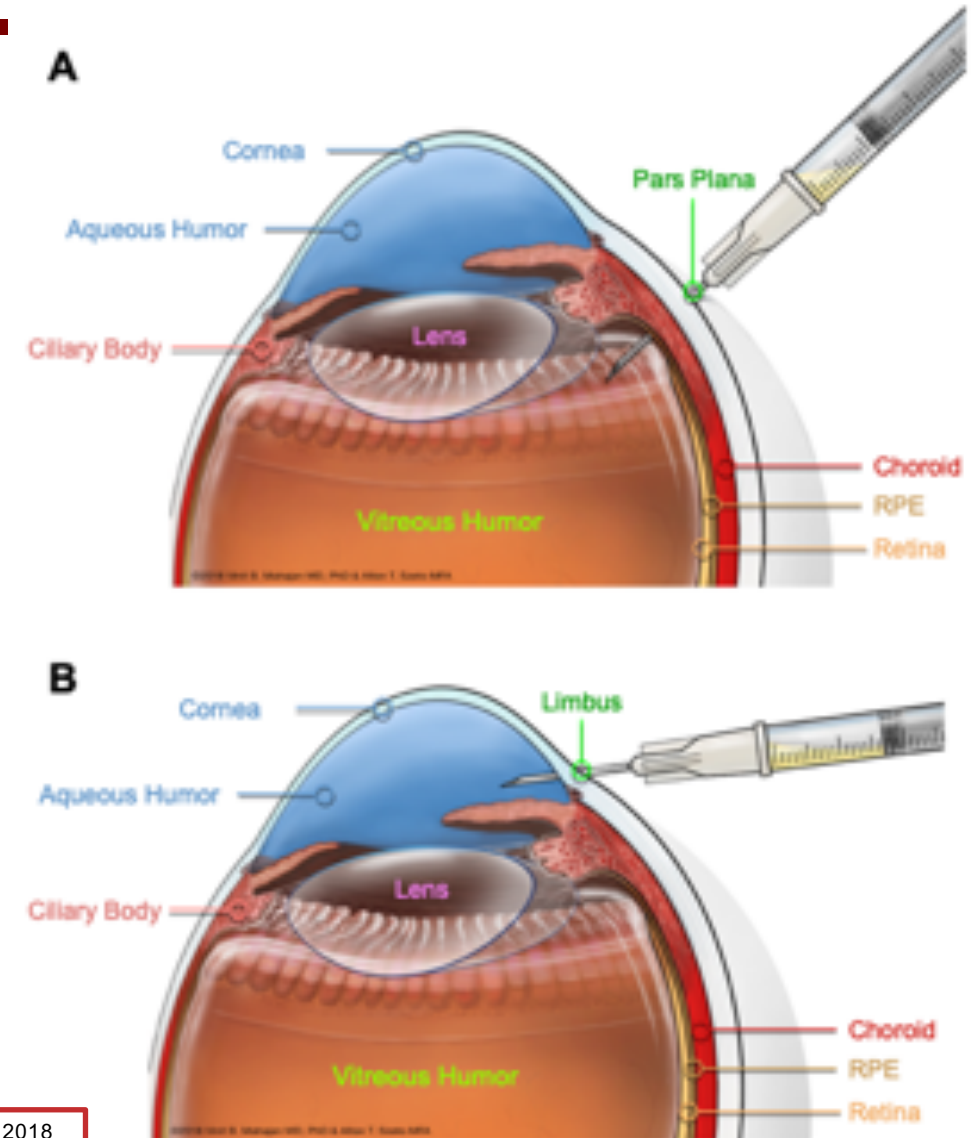
To identify proteomic signatures in UM vitreous that correlate with GEP signatures

• Lacking shotgun approach

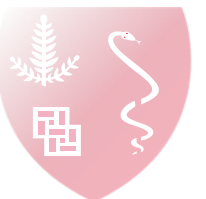
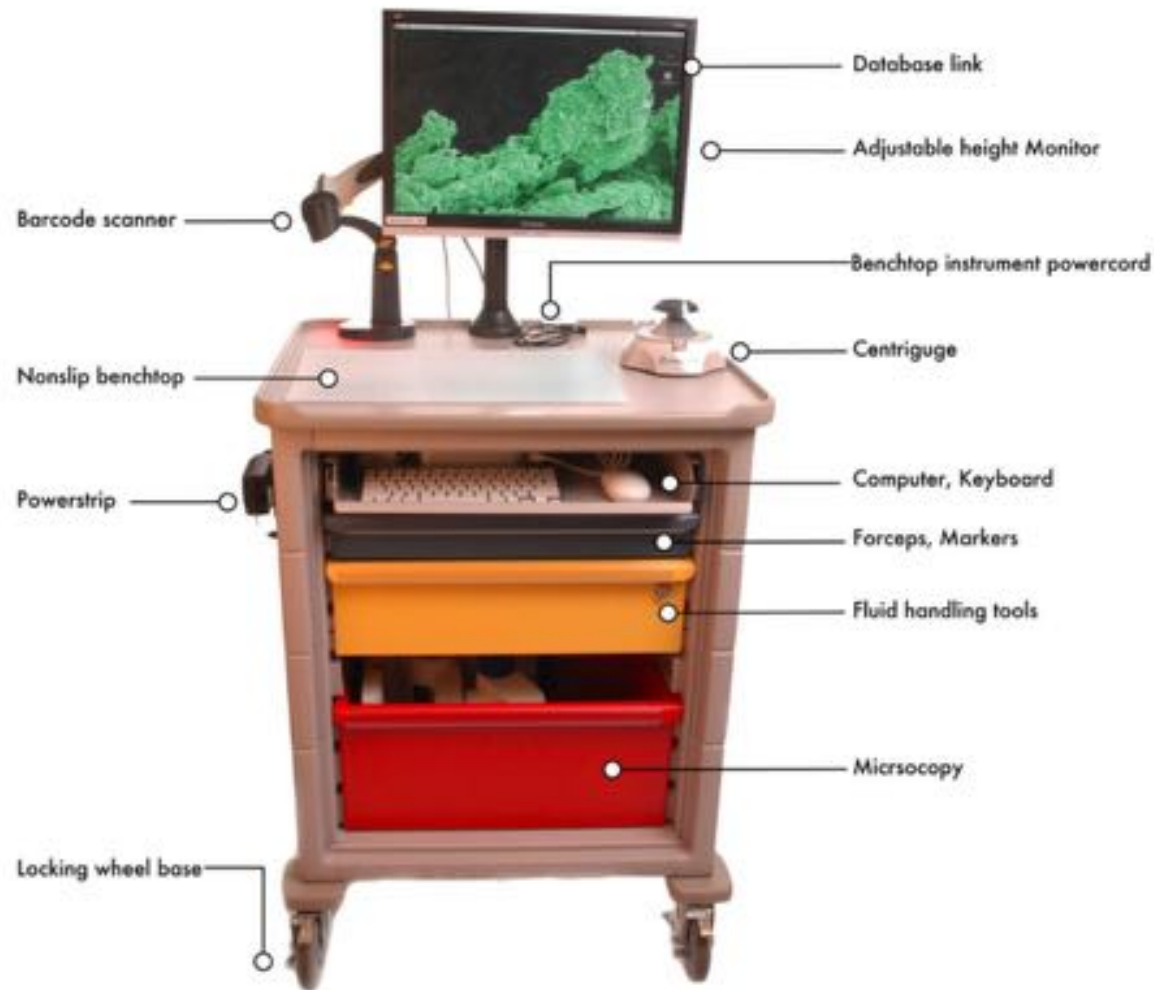


METHODS: IRB APPROVED PROSPECTIVE BIOREPOSITORY STUDY

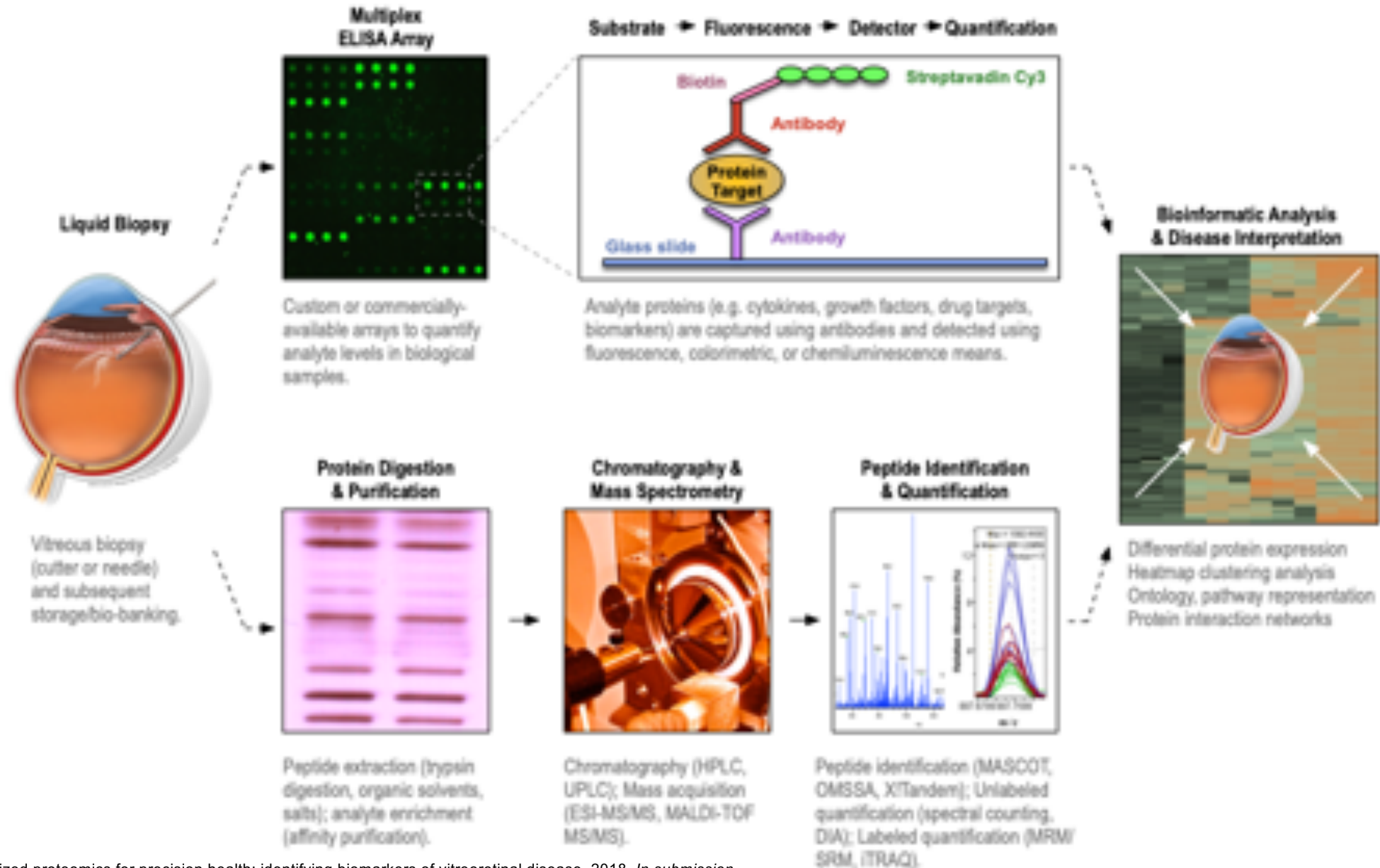
- Plaque placement:
 - 27 g 3-port Diagnostic vitrectomy, aqueous biopsy, tumor sample
- Enucleation
 - Vitreous and aqueous fluid aspiration
- GEP/PRAME
 - Castle Biosciences



MOBILE OR LABORTORY INTERFACE (MORLI)



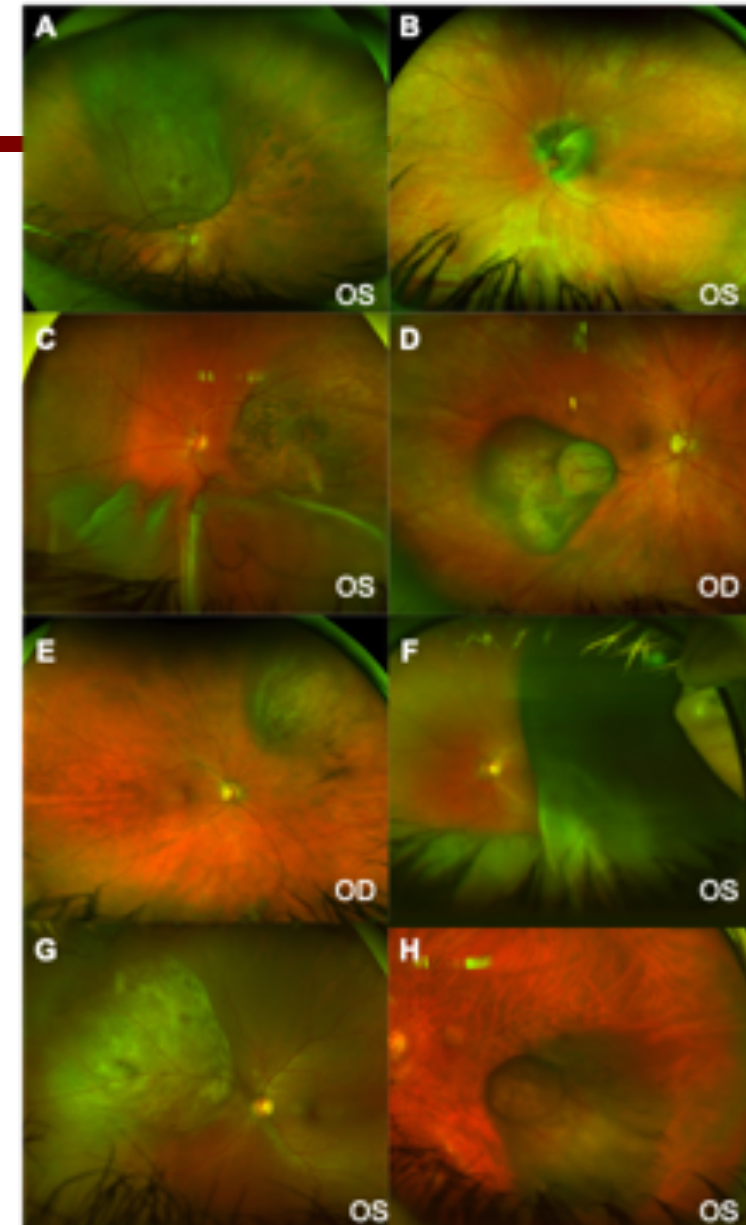
PROTEOMICS PIPELINE FOR PRECISION HEALTH



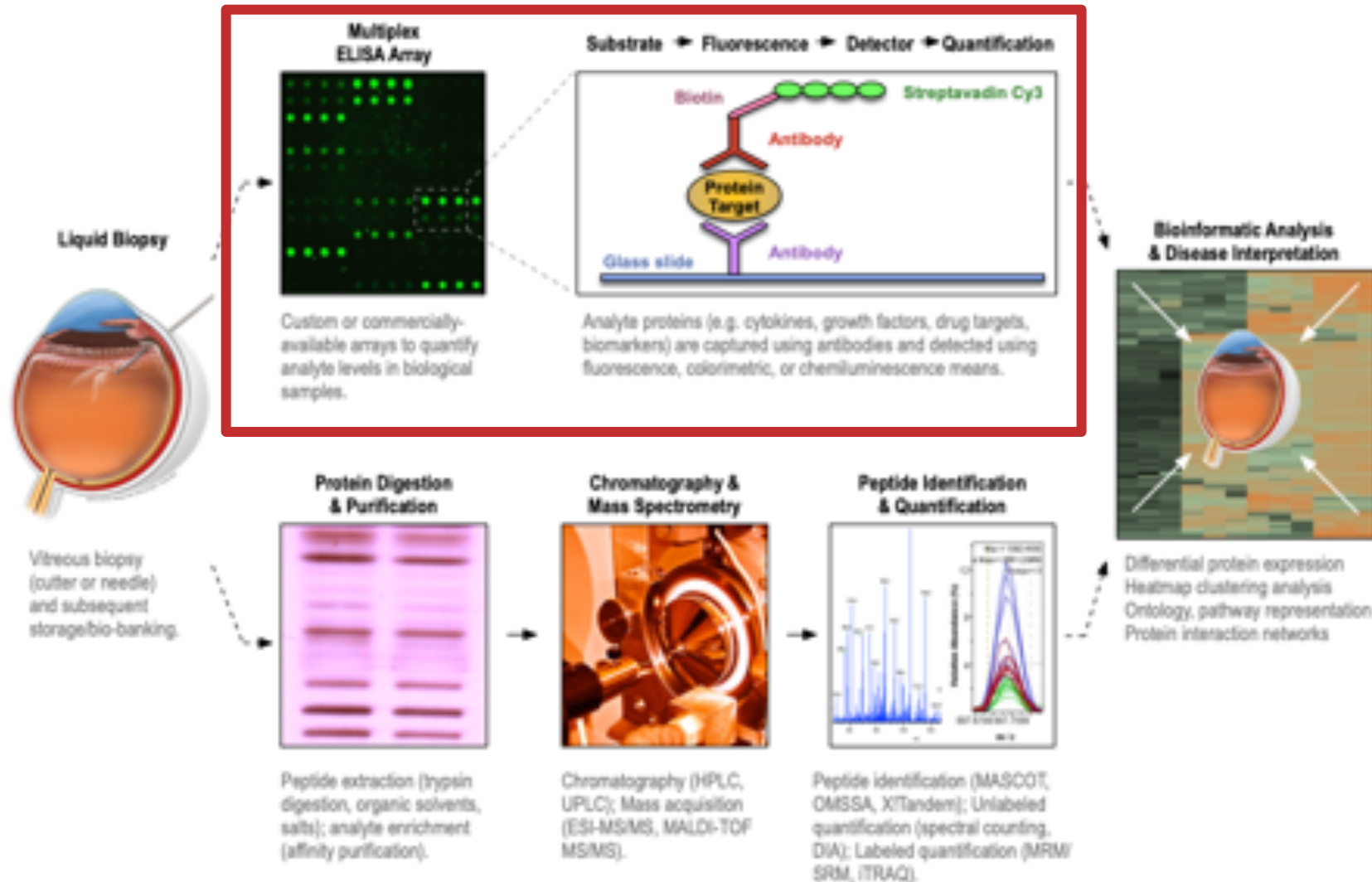
RESULTS: STUDY POPULATION

- 8 subjects with uveal melanoma
 - Mean age 53.9 years.
 - no ciliary body involvement
 - mean thickness 6.6mm.
- GEP :
 - Class 1A (3 eyes), Class 1B (2 eyes), Class 2 (3 eyes)
- PRAME expression was positive in 4 (50%)

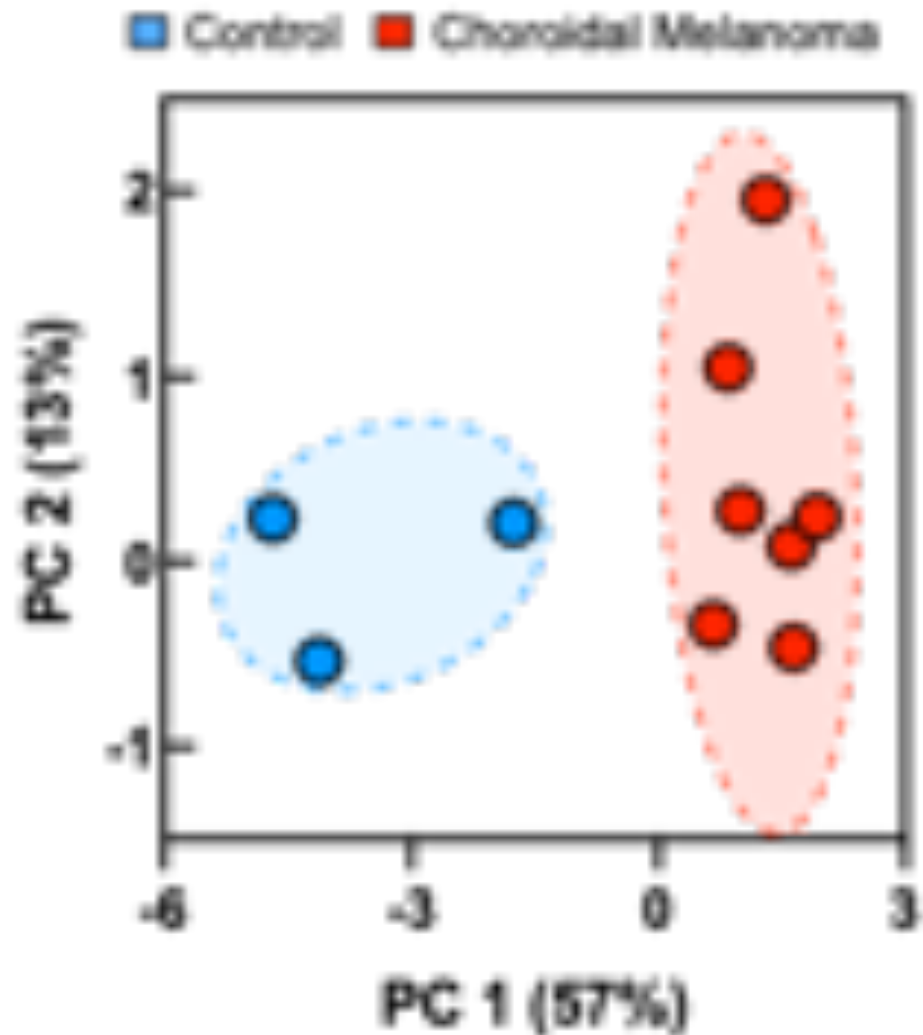
+ 3 CONTROL *ERM* VITRECTOMY SAMPLES



PROTEIN BIOMARKER DISCOVERY PIPELINE



PRINCIPAL COMPONENT ANALYSIS (PCA)



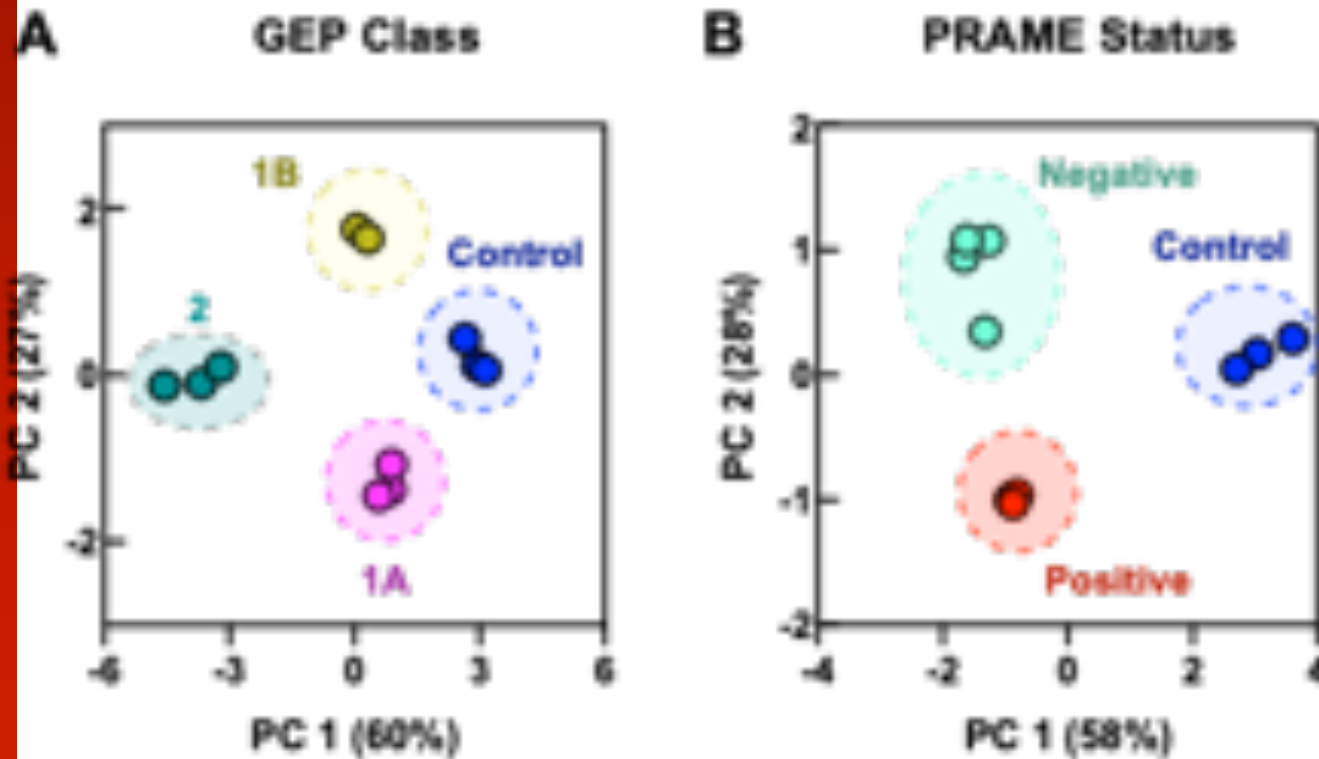
Results:

Protein signatures can distinguish choroidal melanoma from control vitreous.

PROTEIN EXPRESSION BY DISEASE CLASS

Results:

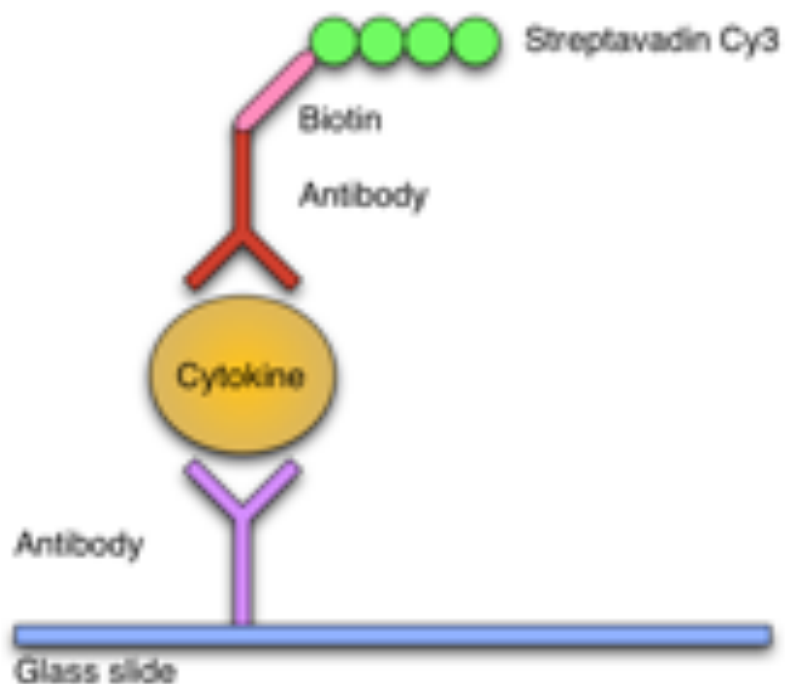
Proteomic signatures can distinguish molecular classes of choroidal melanoma.



TARGETED PROTEOMICS PLATFORMS

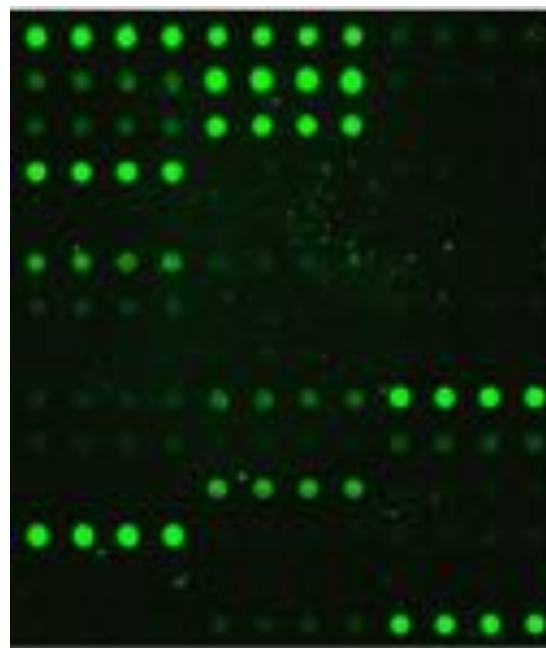
Conventional Test

1 protein



"Proteomics" Test

100-1000 proteins

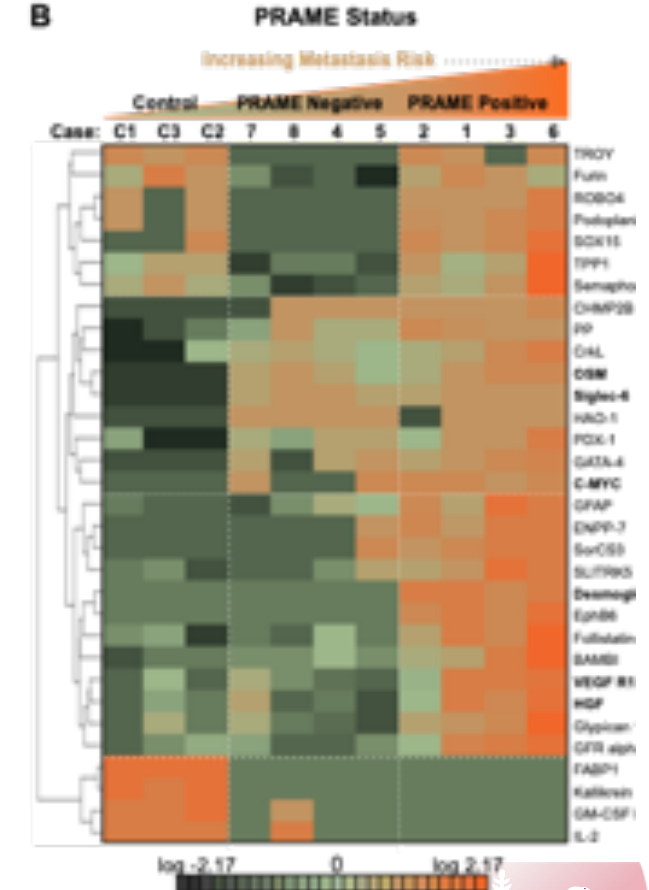
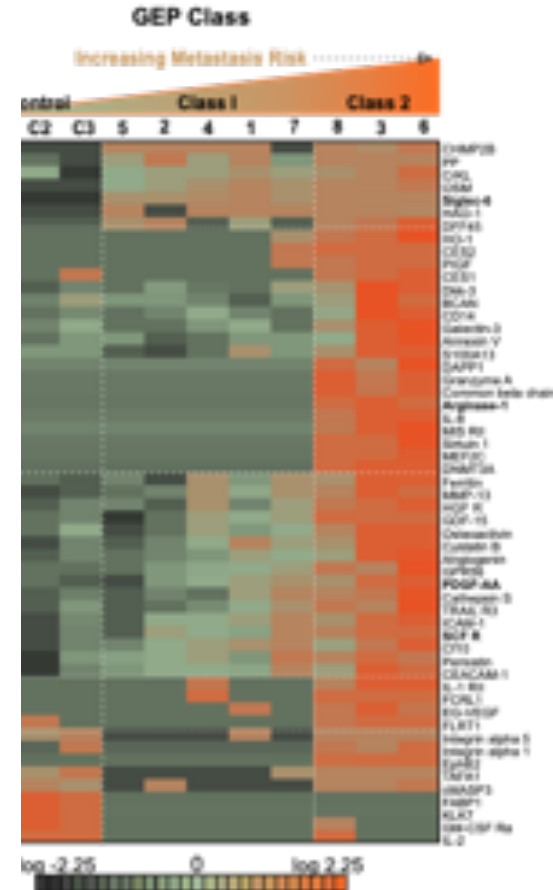


Expression:
62 elevated
15 decreased

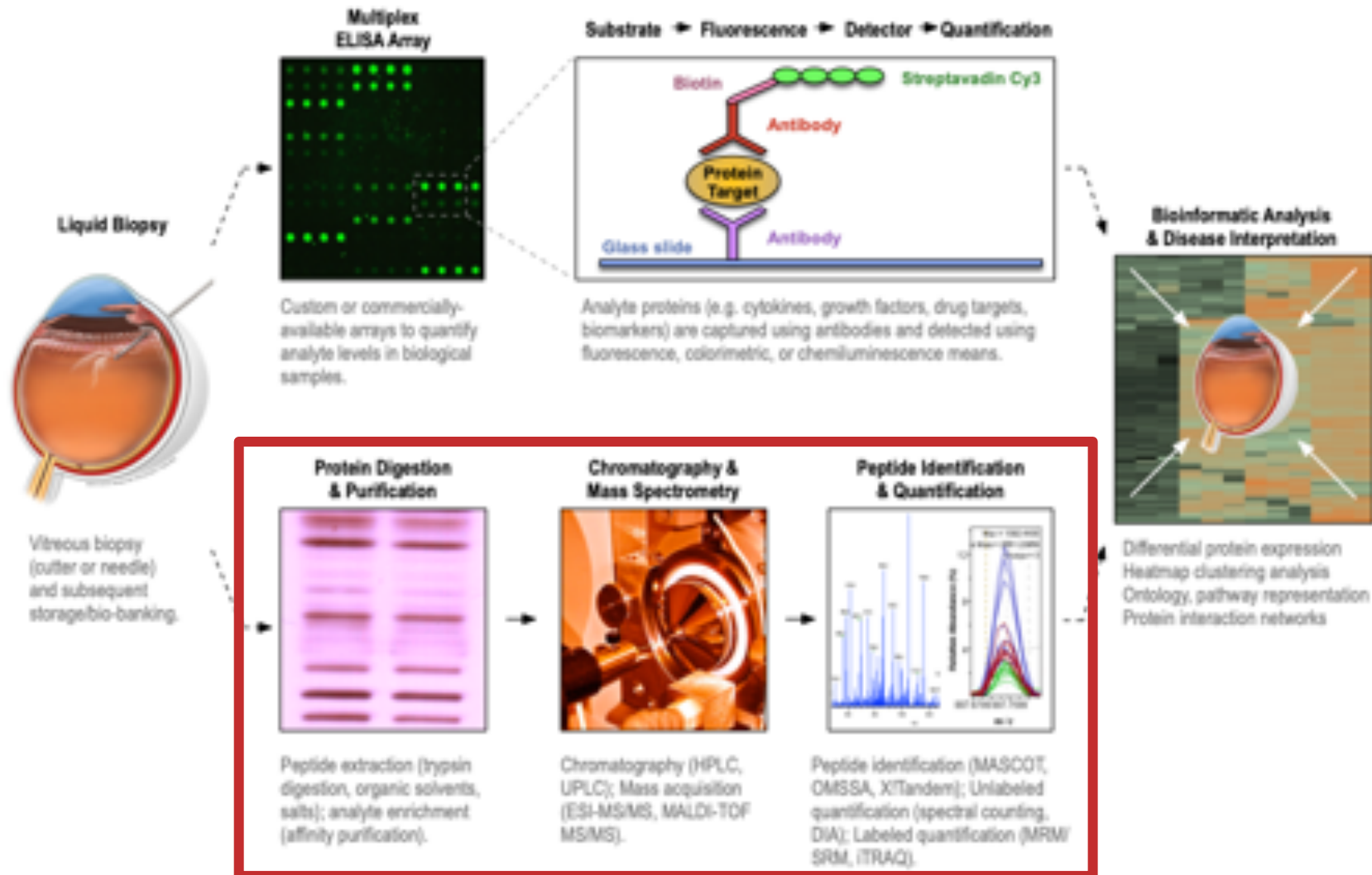
*by multiplex
ELISA*

- GEP class
 - 46 differentially expressed proteins
- PRAME expression
 - 32 differentially expressed proteins

($p < 0.01$)

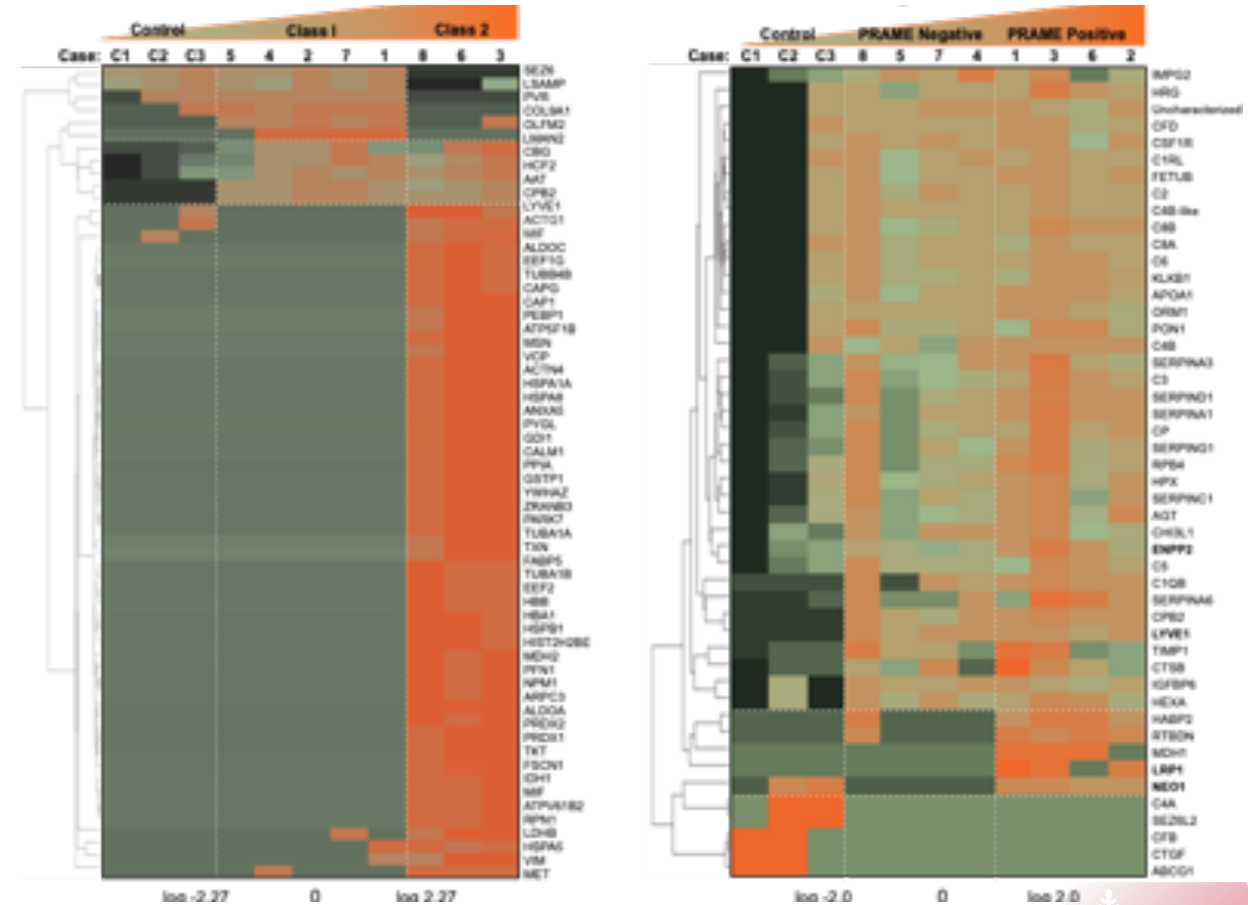


SHOTGUN PROTEOMICS SCREEN



20 CANDIDATE PROTEINS

- Validation in additional 11 UM samples and paired serum
- Identification of GEP and PRAME associated targets



REPURPOSED FOR CHOROIDAL MELANOMA?

- Using protein signatures to target metastatic risk biology with current FDA approved drugs

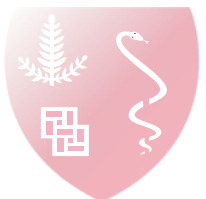
JCI **insight**

CLINICAL MEDICINE

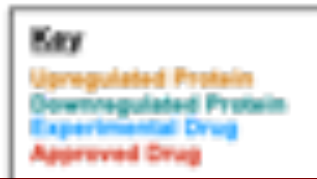
Therapeutic drug repositioning using personalized proteomics of liquid biopsies

**Gabriel Velez,^{1,2,3} Alexander G. Bassuk,⁴ Diana Colgan,^{1,2} Stephen H. Tsang,^{5,6}
and Vinit B. Mahajan^{1,2,3}**

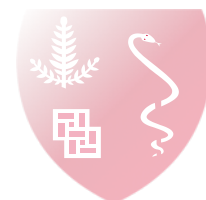
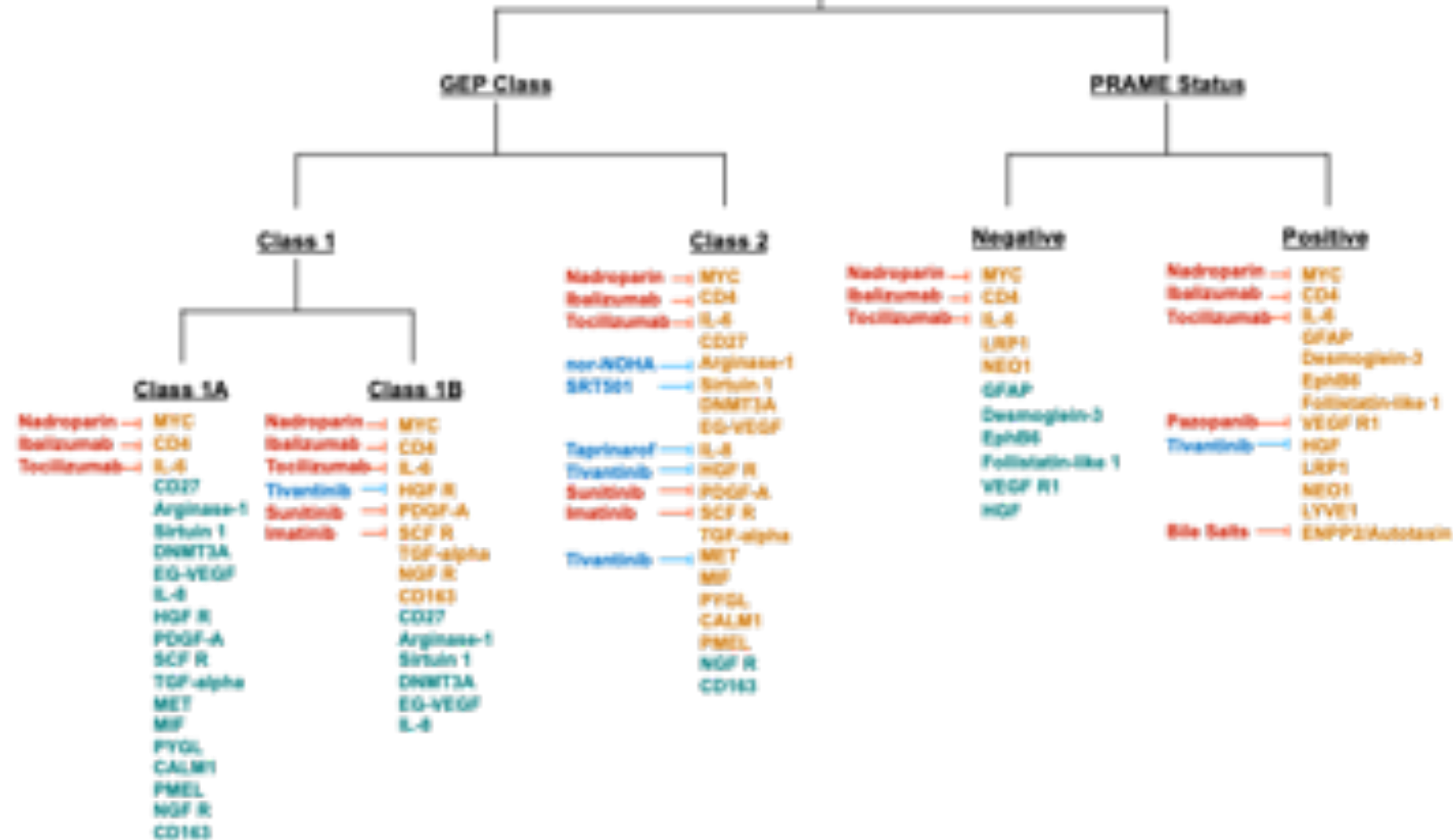
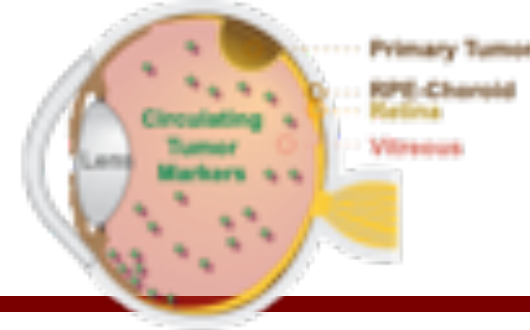
¹Oncics Laboratory, Stanford University, Palo Alto, California, USA. ²Department of Ophthalmology, Byers Eye Institute, Stanford University, Palo Alto, California, USA. ³Medical Scientist Training Program, and ⁴Department of Pediatrics, University of Iowa, Iowa City, Iowa, USA. ⁵Barbara and Donald Jonas Laboratory of Stem Cells and Regenerative Medicine and Bernard & Shifree Brown Glaucoma Laboratory, Edward S. Harkness Eye Institute, and ⁶Department of Pathology & Cell Biology, College of Physicians & Surgeons, Columbia University, New York, New York, USA. ⁷Palo Alto Veterans Administration, Palo Alto, California, USA.



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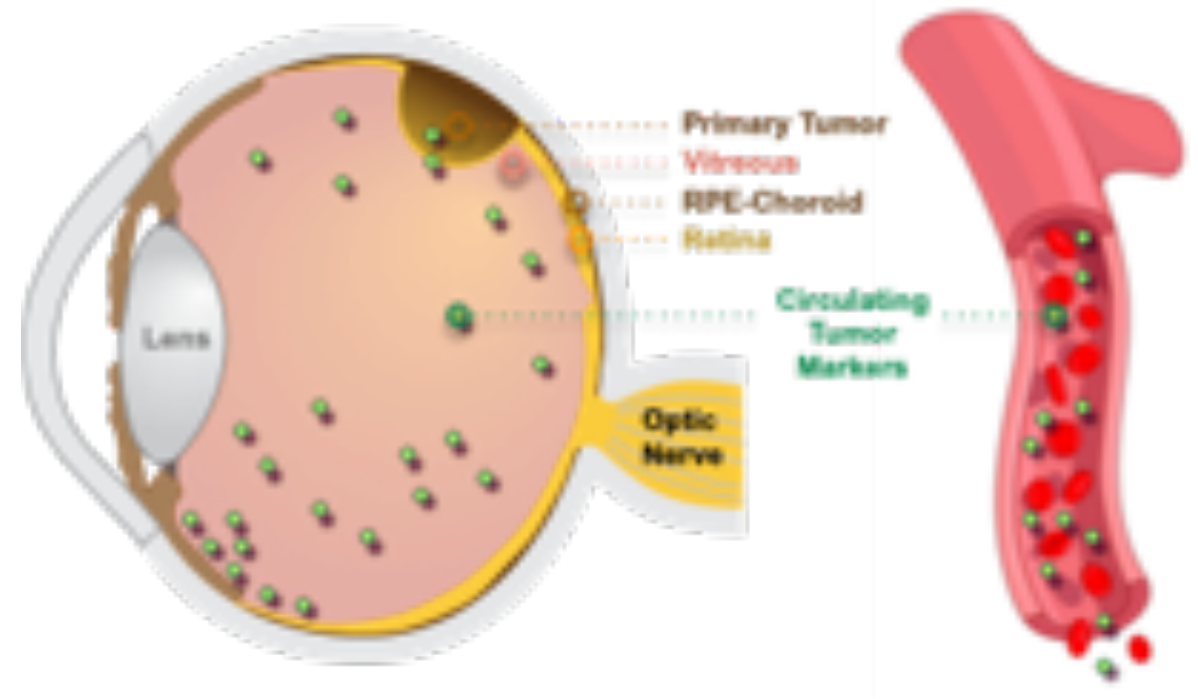
Choroidal Melanoma
↓
Molecular Testing



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FUTURE DIRECTIONS: MELANOMA PROTEOMICS

- Expanded validation
- Serial serum and metastatic tumor
- Plausibility of repurposed agents in adjuvant trials



STANFORD OCULAR ONCOLOGY SERVICE



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OPHTHALMOLOGY
BYERS EYE INSTITUTE



Orbital
Eyelid
Tumors

Ocular
surface
Tumors

Pediatric
Tumors
Retinoblastoma

Intraocular
Tumors
Melanoma

Systemic
cancers and
the eye

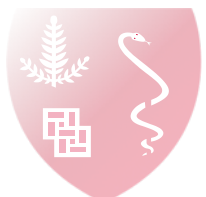
Prithvi Mruthyunjaya, MD, MHS *Director*

Andrea Kossler, MD *Director, Orbital Oncology*

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Albert Wu, MD

ocularoncology@stanford.edu



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